350-Pos
Activation of a Helicase Motor Upon Encounter With a Specific Sequence in the DNA Track
Joseph Yeeles[1], Emma Longman[1], Fernando Moreno-Herrero[2], **Mark S. Dillingham**[1].
[1]University of Bristol, Bristol, United Kingdom, [2]Centro Nacional de Biotecnologia, Madrid, Spain.
DNA helicases are ATP-driven motor proteins that catalyse the separation of duplex DNA into its component single-strands. These are key intermediates in many cellular DNA transactions including replication, recombination and repair. In this study, we have investigated the kinetics of DNA translocation and unwinding by the AddAB helicase-nuclease; an enzyme which is involved in the initiation of double-stranded DNA break repair by homologous recombination. We show that AddAB displays extremely rapid and processive DNA unwinding activity powered by a single Superfamily 1A helicase domain. Uniquely, the helicase activity of AddAB is dramatically stimulated upon encounter in cis with a specific regulatory sequence (Chi) embedded in the DNA track. The molecular basis for this activation of helicase activity and its implications for our understanding of helicase mechanisms in general will be discussed.

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A Closer Look At the Unwinding Initiation By Twinkle-The Human Mitochondrial DNA Helicase
Doyel Sen, Smita Patel.
Robert Wood Johnson Medical School, Piscataway, NJ, USA.
TWINKLE (T7gene4 protein like protein with intra-mitochondrial nucleoid localization) is a hexameric helicase involved in the maintenance of the mitochondrial genome in mammalian cells. It is encoded by PEO1 gene of chromosome 10. Mutations in PEO1 have been linked to mitochondrial DNA (mtDNA) deletions, which lead to various neuromuscular disorders. TWINKLE shares about 46% amino acid sequence similarity with T7gene4 protein (gp4), the bifunctional primase-helicase of bacteriophage T7. Even though T7gp4 is one of the best studied helicases, studies on TWINKLE are only in its nascent stage. In this study, we have purified TWINKLE to near homogeneity and have used it to study the kinetics of dsDNA unwinding by this helicase. We have focused on finding the optimal NTP and DNA substrate for unwinding by TWINKLE. Our studies show that UTP is the preferred substrate for fork DNA unwinding by TWINKLE in vitro. Fork DNA unwinding assays with a variety of 3′-tails indicate that the morphology of 3′-tail is crucial for unwinding by TWINKLE. This is a step towards understanding the mechanism of initiation of replication from the D-loop in the mitochondrial DNA. Our ultimate goal is to understand the mechanism of replication of mitochondrial DNA in vitro which in turn would enable us to define the molecular basis of various diseases associated with mutations in TWINKLE.

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Stepping Mechanism of Bacteriophage T7 Helicase and Priming Loop Observed Using Single-Molecule Fret Methods
Salman Syed[1], Manjula Pandey[2], Smita S. Patel[2], Taekjip Ha[1,3].
[1]Howard Hughes Medical Institute, Urbana, IL, USA, [2]Department of Biochemistry, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ, USA, [3]Department of Physics and the Center for the Physics of Living Cells, University of Illinois, Urbana, IL, USA.
Bacteriophage T7 gp4, a hexameric ring-shaped helicase, serves as a model protein for replicative helicases. T7 helicase couples dTTP hydrolysis to directional movement and DNA strand separation. Previous studies have shown that its DNA unwinding rate and dTTPase rate are sensitive to base pair (bp) stability.We confirm that the unwinding rate of T7 helicase decreases with increasing base pair (bp) stability. For duplexes containing $>$ 35% GC basepairs, we observed stochastic pauses during unwinding. Using an AT block followed by GC block as a calibration tool, we determine that the pausing occurs every 2-4 bp, regardless of detailed sequence composition. The dwells on each pause were distributed non-exponentially, consistent with several rounds of dTTP hydrolysis events before each unwinding step.
We also study the dynamics of the T7 replisome. We show that in the presence of T7 DNA polymerase, the primase does not slow down or pause the T7 helicase during primer synthesis. Furthermore, we observe a formation and disappearance of a ssDNA loop between the helicase and the primase domains during the unwinding/leading strand synthesis. Our results suggest a model whereby the priming loop allows the coordination of leading and lagging strand DNA synthesis by keeping the primer in physical proximity to the replication complex by assuring efficient primer utilization and hand-off to the polymerase.

353-Pos
Direct Observation of Twisting Steps During Rad51 Polymerization on DNA
Giovanni Cappello[1], Hideyuki Arata[1], Aurélie Dupont[1], Ludovic Disseau[1], Jean-Louis Viovy[1], Axelle Renodon-Cornière[2], Masayuki Takahashi[2].
[1]Institute Curie, Paris Cedex 05, France, [2]Université de Nantes, Nantes cedex 3, France.
The human recombinase hRad51 is a key protein for the maintenance of genome integrity and for cancer development. This protein plays a central role is the DNA strand exchange occurring during homologous recombination. Here we report the polymerization and depolymerization of hRad51 on duplex DNA observed with a new generation of magnetic tweezers, allowing the measurement of DNA twist with a resolution of 5° in real time. At odds with earlier claims, we show that, after initial deposition of a multimeric nucleus, nucleoprotein filament growth occurs by addition of single proteins, involving DNA twisting steps of $65 \pm 5°$. Simple numerical simulations support that this mechanism is an efficient way to minimize nucleoprotein filament defects. This behavior, consisting of different stoichiometry for nucleation and growth phases, may be instrumental in vivo. Fast growth would permit efficient continuation of strand exchange by Rad51 alone while the limited nucleation would require additional proteins such as Rad52, thus keeping this initiation step under the strict control of regulatory pathways. Besides, our results combined with earlier structural information, suggest that DNA is somewhat less extended (4.5 versus 5.1 Å per bp) than by RecA, and confirm a stoichiometry of 3-4 bp per protein in the hRad51-dsDNA nucleoprotein filament.

354-Pos
Real-Time Visualization of Assembly and Disassembly of S. Cerevisiae Rad51 on Duplex DNA
Ichiro Amitani, Amitabh V. Nimonkar, Ronald J. Baskin, Stephen C. Kowalczykowski.
University of California, Davis, Davis, CA, USA.
DNA double-strand break (DSB) is a lethal type of DNA damage. Homologous recombination is the most accurate method to repair broken DNA. The process of recombinational DNA repair consists of three phases. First, the ends of broken DNA are processed to generate a 3′-ended single-strand DNA (ssDNA) tail on which the DNA strand exchange protein self-assembles. Second, the protein-DNA complex searches for homology on a donor double-strand DNA (dsDNA), and catalyzes the pairing and exchange of DNA strands. Finally, the heteroduplex DNA product is resolved.
In S. cerevisiae, DNA strand exchange is catalyzed by Rad51 protein. Rad51 forms a right-handed helical nucleoprotein filament in an ATP-dependent manner. In the filament, Rad51 occupies 3-4 nucleotides and stretches DNA to ~150% of its B-form length. Interestingly, Rad51 binds to dsDNA with a similar affinity as for ssDNA, even though the functional form of Rad51 requires assembly on ssDNA. Also, since Rad51 filament formation on dsDNA inhibits DNA strand exchange, the turnover of Rad51 from dsDNA is stimulated by a dsDNA translocase, Rad54.
We used single-molecule fluorescence microscopy to visualize both the kinetics of assembly and disassembly of Rad51 nucleoprotein filaments. Visualization was achieved either by directly imaging filament formation using fluorescent Rad51 protein, or by measuring the DNA length change due to Rad51 binding by fluorescently tagging the DNA end. We show that the nucleation of Rad51 on dsDNA requires about 3 monomers; that Rad51 assembly occurs through frequent nucleation; and that the Rad51 filament is stabilized by maintaining ATP-bound state.

355-Pos
Coupling DNA Unwinding Activity With Primer Synthesis in the Bacteriophage T4 Primosome
Vincent Croquette[1], Maria Manosas[1], Michelle M. Spiering[2], Zhihao Zhuang[3,4], Stephen J. Benkovic[3].
[1]LPS-ENS-CNRS, Paris, France, [2]Department of Chemistry, The Pennsylvania State University,, PA, USA, [3]Department of Chemistry, The Pennsylvania State University, PA, USA, [4]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE, USA.
The unwinding and priming activities of the bacteriophage T4 primosome, which consists of a hexameric helicase (gp4) translocating 5′ to 3′ and an oligomeric primase (gp6) synthesizing primers 5′ to 3′, have been investigated on DNA hairpins manipulated by a magnetic trap. We find that the T4 primosome continuously unwinds the DNA duplex while allowing for primer synthesis through a primosome disassembly mechanism or a new DNA looping mechanism. A fused gp6-gp4primosome unwinds and primes DNA exclusively via the DNA looping mechanism. Other proteins within the replisome control the partitioning of these two mechanisms, which disfavor